BRANCHIAL RESPONSES TO ADRENALINE AND TO PITRESSIN IN THE EEL

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INTRODUCTION

The gill perfusion method (Keys, 1931*a*) affords a convenient means of investigating the physiology of the branchial blood vessels in the fishes. In particular, it would seem to be desirable to study the effects of various hormones on the effective calibre of the branchial vessels and on the performance of the branchial chloride-secreting mechanism (Keys, 1931*b*).

We have investigated the effects of adrenaline and of pitressin on the gills of the eel, *Anguilla vulgaris,* by means of the ventral aorta-gill preparation as described in a recent paper (Bateman and Keys, 1932). Throughout each experiment the perfusion pressure (mean of systole and diastole of the pump) was maintained at a constant level by adjustment of the pump stroke and the reservoir level.

Rates of perfusion were measured from the rates of inflow in some of the experiments and from the rates of outflow from the dorsal aorta in the other experiments. In the latter cases the dorsal aorta was cannulated at the level of the anterior portion of the liver. Practically identical results were obtained from these two methods. Venous escape from the cardinal and coronary systems was prevented in all cases except where specifically mentioned.

The perfusion fluid used was the same as that given by Keys (1931*a*, p. 359), the concentrations being $\Delta =$ about 0.72° for eels from sea water and $\Delta =$ about 0.60° for eels from fresh water. The external medium was either Plymouth sea water or Cambridge tap water, depending upon the medium in which the eels had been kept prior to the experiment.

The net chloride exchange between internal and external media in the gills was determined by analyses of ingoing and outgoing fluids, using Keys' (1931*c*) method. Analyses were done in duplicate or triplicate.

The effects of adrenaline were determined by the addition of adrena-

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line chloride (Parke Davis) to the perfusion fluid immediately before use so as to give adrenaline concentrations between 1/300,000 and 1/1,000,000. Pitressin (Parke Davis), the pressor principle from the posterior lobe of the pituitary gland, was used in concentrations ranging

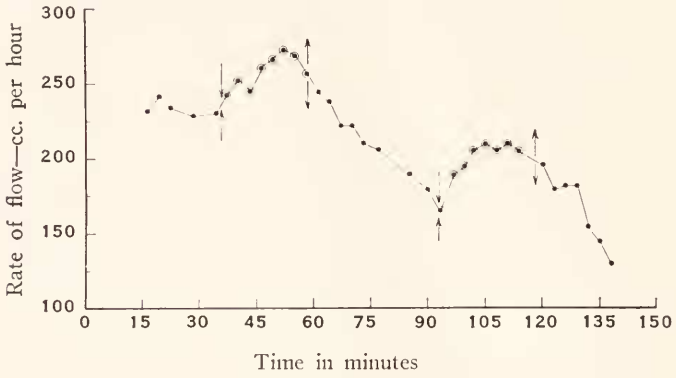

FIG. 1. The influence of adrenaline (1/1,000,000) on the rate of flow through the gills under constant pressure. The encircled points are from measurements during adrenaline perfusion, and the arrows indicate the introduction and removal of adrenaline.

from 25 to 50 international pressor units per liter by addition to the perfusion fluid just prior to use. The normal perfusion fluid was used as control in all cases.

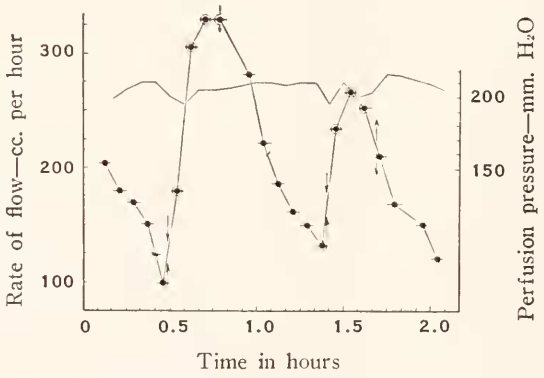

FIG. 2. The influence of adrenaline (1/500,000) on the rate of flow through the gills under constant pressure. The line drawn through the points shows the rate of flow. The encircled points are from measurements during adrenaline perfusion, and the arrows indicate the introduction and removal of adrenaline The continuous line shows perfusion pressure.

The Effect of Adrenaline on the Calibre of the Branchial Vessels

Adrenaline, in the concentrations used here, provoked unmistakable dilatation of the branchial vessels. Figures 1 and 2 show the pronounced increase in rate of flow through the vessels resulting from the addition of adrenaline to the perfusion fluid.

The experimental results shown in Fig. 1 and Fig. 2 are typical. The results of additional experiments are shown in Table I in which mean relative values for rates of flow with and without adrenaline are given. In each case the initial rate of flow without adrenaline is taken as 100 and all subsequent rates of flow expressed as percentage of the initial value.

TABLE I

Effect of adrenaline on the mean rate of flow through the perfused branchial vessels. All rates of flow expressed as percentage of the initial value.

Adrenaline concentration	Rate flow without	No. determinations	Rate flow with	No. determinations	Rate flow without	No. determinations	Rate flow with	No. determinations	Rate flow without	No. determinations	Rate flow with	No. determinations
1/300,000	100	3	143	10	128	11						
1/500,000	100	9	172	4	110	5	160	3	96	4		
1/500,000	100	6	116	10	91	9	96	6	75	7		
1/700,000	100	3	123	4	110	4	133	5	111	5	131	4
1/1,000,000	100	5	112	7	103	4						

Adrenaline in these concentrations produces vasoconstriction in the vessels (other than the coronaries) of mammals. However, adrenaline in some concentrations (about 1/20,000,000) is known to have a small true vasodilator action (Dale and Richards, 1927) and it was thought necessary to test the effect on systemic vessels of the eel of the adrenaline concentrations used in the gill perfusions.

A preparation was made with the posterior part of the eel perfused from the dorsal aorta. The adrenaline effect was entirely similar to that obtained with the systemic vessels of the mammals, very pronounced vasoconstriction being obtained with adrenaline concentrations of 1 500,000 and 1/1,000,000. Figure 3 gives the results of an experiment of this type.

The final proof of the dissimilarity of the action of adrenaline on the branchial vessels and on those of the systematic circulation is provided by the following experiment. An eel is pithed and secured to the oper-

ating board. A mid-line incision is made exposing the heart and the ventral aorta. The sinus venosus is opened and the ventral aorta cannulated so that the course of the perfusion is through the gills to the systemic circulation and finally out through the opening in the sinus venosus. If, now, adrenaline is added to the perfusion fluid and the perfusion pressure is kept constant there is an almost immediate great reduction in the rate of flow, and removal of the adrenaline from the perfusion fluid restores the perfusion rate as soon as the adrenaline has been washed out of the system.

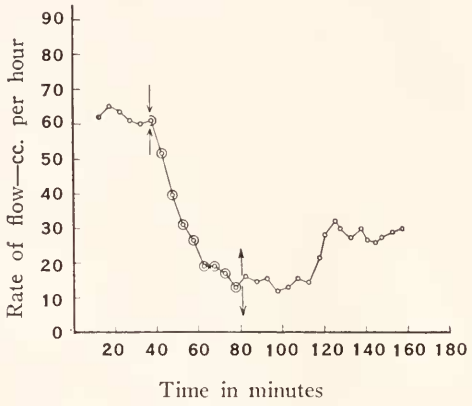

FIG. 3. The influence of adrenaline (1/500,000) on the rate of flow through the systemic vessels of the posterior part of the eel under a constant perfusion pressure of 35 cm. H_2O. The encircled points are from measurements during adrenaline perfusion and the arrows indicate the introduction and removal of adrenaline.

The effect of the adrenaline on the gills alone is demonstrated on the same preparation simply by cutting through the dorsal aorta at the level of the sinus venosus so that the perfusion is short-circuited past the systematic circulation. The results of this experiment are shown in Fig. 4.

THE EFFECT OF ADRENALINE ON THE CHLORIDE SECRETION

The apparent chloride secretion was always depressed by adrenaline in the experiments in which chloride concentrations were measured. The results of the two most complete experiments of this type are shown in Fig. 5 and Fig. 6.

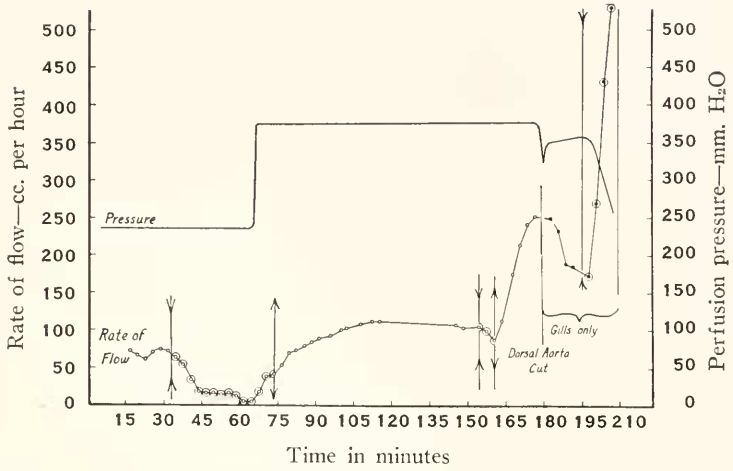

FIG. 4. A comparison of the adrenaline effect on the systemic and branchial vessels of the eel. The encircled points are from measurements of rates of flow during adrenaline perfusion and the arrows indicate the introduction and removal of adrenaline. See text for details of the experiment.

It will be noted that the adrenaline vasodilatation is very evident and closely parallels the very pronounced diminution in the chloride secretion. Figure 6 is particularly instructive; under the influence of adrenaline the secretion not only disappeared but the internal fluid became more concentrated during its passage through the gills. Table II, containing the condensed protocol of this experiment, will make clear the details of the method of calculation employed.

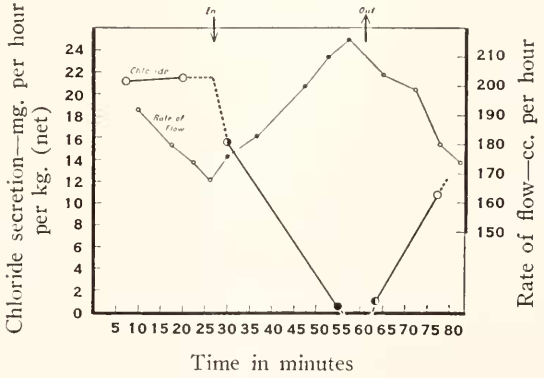

FIG. 5. The effect of adrenaline on chloride secretion in the gills bathed externally with sea water. Concentration of adrenaline, 1 in 1,000,000.

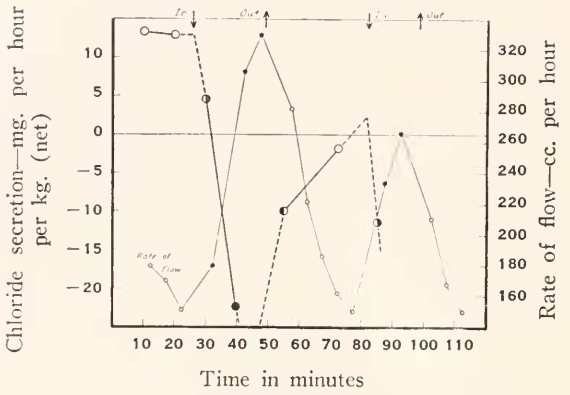

FIG. 6. The effect of adrenaline on chloride exchange in gills bathed externally with sea water. Concentration of adrenaline, 1 in 500,000.

The mean chloride change for all the experiments was 14.5 mg. in the periods with normal Ringer's solution and 1.9 mg. in the adrenaline

TABLE II

Protocol of Experiment 2A on the effect of adrenaline on the secretion of chloride by the perfused branchial vessels.

Eel from sea water, weight 370 grams. External medium, sea water; Δ = 2.0°. Internal medium (1) normal; eel Ringer's solution; Δ = about 0.72°. (2) adrenaline; same as (1) but plus adrenaline chloride to give final adrenaline concentration of 1/500,000.

Internal medium	Time of collection of perfusate and control		Mean perfusion pressure	Mean rate flow	Chloride concentrations		Difference chloride	Net chloride change
	beginning	end			control	expt.		
			mm. Hg	*cc./hr.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./hr./kg. eel*
Normal	1:05	1:15	20	202	693.0	691.4	−2.6	−13.4
Normal	1:15	1:25	21	160	693.0	690.0	−3.0	−13.0
Adren. (in gills about 1:31)	1:25	1:35	19	189	692.6	691.7	−0.9	− 4.6
Adrenaline	1:35	1:45	20	318	692.4	696.0	+2.6	+22.3
Normal (adr. out about 1:50)	1:45	2:05	20	278	693.8	695.1	+1.3	+ 9.8
Normal	2:05	2:20	20	166	693.5	693.7	+0.2	+ 0.9
Adrenaline (in gills about 2:25)	2:20	2:30	19	183	693.1	695.4	+2.3	+11.4
Adrenaline	2:30	2:40	19	259				
Normal (adr. out about 2:45)	2:40	2:50	21	189				
Normal	2:50	3:05	20	135				

periods, these figures representing the net loss of chloride from the internal medium per hour and per kilogram of eel. These values give really a minimum estimate of the difference between the apparent secretion in the two conditions owing to the fact that there was a considerable amount of overlap in many of the collections. In almost all cases the first period of collection of perfusate following a change in the perfusion medium represented, in reality, a mixture of perfusate from the adrenaline and the normal perfusion fluids.

The Effects of Pitressin on the Gills

The experiments with the pressor principle ("pitressin," Parke Davis) of the pituitary gland demonstrated only that this hormone, in the concentrations we used, has little effect either on the mean calibre of the branchial vessels or on the branchial chloride secretion.

In four experiments involving eight periods of normal perfusion fluid and six periods of pitressin (25 to 50 units per liter), the effect of the pitressin on the rate of flow was slight and variable. The maximum effect observed was a mean *decrease* of 17 per cent; the next greatest effect was an *increase* of 10 per cent. The average of all the measurements of rate of flow was very nearly the same with the normal perfusion fluid and with the pitressin. Taking the average of the non-pitressin periods as 100, the average for the pitressin periods was 97.

The chloride secretion by the gills appeared to be almost unaffected by pitressin and such effects as were observed were variable from one experiment to the next. In two experiments there was an increase in the chloride secretion during pitressin but in two other experiments the effect was in the opposite direction. In all cases the effect was small. The maximum change in the net chloride secretion during pitressin was an increase of 5.3 mg. Cl per hour and per kilogram of eel; the next greatest effect observed was a decrease of 4 milligrams.

Discussion

Wyman and Lutz (1932) have studied the effects of adrenaline on the blood pressure of the elasmobranch, *Squalus acanthias*. They recorded dorsal and ventral aorta blood pressures and found sustained pressor effects even with very small doses of adrenaline, which they interpreted ". . . as being due to extra-cardiac factors, peripheral to the gill capillaries, but the region of action of the adrenaline was not located." [3]

[3] Strictly, there are no true capillaries in the gills; see Keys and Willmer (1932) for the structure of the branchial vessels.

These results are obviously in complete agreement with our findings and we feel safe in predicting that suitable experimental technique would reveal adrenaline vasodilatation in the gills of the elasmobranch.

Since the completion of the present experiments we have discovered an interesting paper by Krawkow (1913) which apparently has escaped notice by other workers. Krawkow removed the gills of the pike and cannulated the stump of the ventral aorta. He estimated vasodilatation and constriction by perfusing and counting the number of drops from the cut ends of the gill bars, and he studied the effects of various substances with this technique. His results may be summarized in his own words (p. 603): "Von den untersuchten Substanzen bewirken Imidazolyläthylamin, Nikotin und Chlorbaryum Verengerung der Kiemengefässe. Coffein bewirkt nach kurzdauernder geringer Verengerung bedeutende Erweiterung der Gefässe. Chloroform erweitert die Kiemengefässe. Adrenalin, sogar in starken Verdünnungen, bewirkt sehr bedeutende Erweiterung die Kiemengefässe."

It would seem that the vasodilator effect of adrenaline in the gills of fishes is general. The similarity of this response to the adrenaline vasodilatation of the coronary vessels will have been noted. There is no longer any question as to the adrenaline vasodilatation of the coronary vessels (see Anrep, 1926, for literature), and Rössler and Pascual (1932) have shown that this is a direct response and is not due to an accumulation of metabolites. The latter possibility, of course, is eliminated in perfusion experiments like the present.

It is well known that the coronary arteries of the fish heart arise chiefly from the ventral ends of the posterior branchial vessels (Cuvier, 1805, and many subsequent workers,—see Grant and Regnier, 1926, p. 293) and, in fact, may be considered to be outgrowths of the branchial vascularization. Grant and Regnier (*op. cit.*) have assembled evidence to show that the coronaries in all probability are homologous throughout the vertebrates, ". . . the only important point of difference being the remote branchial origin in the lower vertebrates," (p. 294).

It does not seem to be unwarranted to suppose that the original specialization of vessels which respond to adrenaline by dilatation rather than constriction occurred in the gills of the early fishes. The gill most certainly began to evolve before the coronaries and it is difficult to see how an animal could survive if the vessels of its gills were subject to constriction at those critical moments of danger when adrenaline is liberated into the blood stream. If this view is correct, then the vasodilatory response to adrenaline characteristic of the coronary arteries of the higher vertebrates represents a useful heritage of the primitive condition in the gills.

There are two possible explanations of the effect of adrenaline on the chloride secretion. It may be, of course, that adrenaline simply paralyzes the secretory mechanism; the increase in the concentration of the internal medium during its passage through the gills which is observed in some cases following adrenaline (see Fig. 5) would then result from the normal permeability (Sumner, 1906; Smith, 1930; Keys, 1931*b*) of the gill membranes. This explanation is open to the objection that the indicated permeability may far surpass other estimates of the normal gill permeability (Keys, 1931*b*, p. 378 et seq.).

The alternative explanation for the effect of adrenaline on the chloride secretion is that the vasodilatation greatly increases the permeability so that the passive exchange may exceed the effect of the secretion. Such a relation between dilatation of minute vessels and permeability is apparent from the work of many investigators (see Krogh, 1929, p. 332) and would seem to be quite general. Krogh (*op. cit.*, p. 335) states: "I find no case in which a considerable dilatation has taken place without being accompanied by an increase in permeability."

We have compared the adrenaline response of the gill vessels to that of the coronary arteries; it may be instructive to make a similar comparison with regard to the effect of pitressin. Various workers have studied the effect of pituitary extracts on the calibre of the coronary vessels, but the results have not been consistent. Pal (1909), De Bonis and Suzanna (1909), and Dale (1909) reported vasoconstriction in mammalian coronaries, but Cow (1911) and Rabe (1912) could not confirm these results. Sumbal (1924) reported vasodilatation in the coronaries of the tortoise following administration of "infundin."

It seems that both the coronary vessels and the gill vessels may differ from the systemic vessels in not conforming to the general rule of vasoconstriction with pituitary extracts. It may be mentioned, however, that Drinker (1927) perfused the web of the frog with pituitrin (which is now known to be a mixture of the pressor and oxytocic principles of the pituitary gland) and found little effect on the diameter of the capillaries and no effect on the permeability.

In view of the pronounced effect of pitressin on the secretion of the kidney (Bugbee and Simond, 1928), it is of interest to note that no such effect on the branchial secretion was found in spite of certain other resemblances (Keys, 1931*b*, p. 382 et seq.) between the performance of the two organs.

Summary

Perfusion experiments are described in which the effects of adrenaline and of pitressin on the gills of eels were studied.

It is shown that adrenaline has a marked vasodilator effect on the gills whereas the same concentration of adrenaline has a powerful constrictor effect on the systemic vessels.

Adrenaline was found to decrease or abolish completely the branchial secretion of chloride; this effect is interpreted as being due to increased permeability of the gills associated with vasodilatation.

Pitressin did not appear to have any large or consistent effect either on chloride secretion or on the effective calibre of the branchial vessels.

The evolutionary and physiological relations between the gills and the coronary vessels are discussed. It is suggested that the adrenaline vasodilatation of the coronary arteries may represent a phylogenetic inheritance of a specialized vascular response originally developed in the gills.

Our best thanks are due to Professor Joseph Barcroft for his interest in these studies.

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